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A NEW METHOD TO DISCRIMINATE BETWEEN ENZYME-KINETIC MODELS

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Abstract—A novel statistical procedure [S. Zwanzig, *Math. Opsforsch. Statist. Ser. Statist.* **11**, 23–47 (1980)] is applied to discriminate between kinetic models of enzyme reactions. Two examples are elucidated on the basis of numerically simulated data:

(a) Discrimination between a Michaelis–Menten-model and a Hill-equation
(b) Discrimination between competitive and noncompetitive inhibition of a two-substrate reaction.

Next the proposed method is applied to assess a kinetic model of the allosteric enzyme phosphofructokinase from the malarial parasite *Plasmodium berghei*. It is demonstrated that the novel procedure allows a more sensitive discrimination as it is obtained with alternative statistical tests.

1. INTRODUCTION

The quantitative description of experimental data in enzyme kinetics is based on a suitable model (rate law) which is fitted to the data by nonlinear regression analysis [1–12]. But very rarely is it critically examined whether the chosen model provides a good fit or whether there are alternative models which give a significantly better quantitative representation of the data set (model discrimination [13–15]).

There are several statistical tests which are usually applied in biochemistry to judge the goodness of the fit of the models. For example, Atcins [16] has applied various statistical procedures to select from possible models for an enzyme catalysed reaction the most sufficient one. Landaw and Distefano [17, 18] involved several statistical methods in the analysis of time-series by multi-exponential models. Reich [19] and others [20–23] studied some of the theoretical and conceptual implications of a "good fit" vs a "bad fit". Mannervik and Bártfai [24–26] used nonlinear regression analysis and checked the residuals to discriminate between two rate equations and to optimize the experimental design. Pedersen and Pedersen [27] proposed a method to derive the best binding site model.

Most of the commonly used goodness-of-fit criteria are based on the examination of the residuals $\Delta y = y_i - g(x_i)$, i.e. the differences between the observed data y_i and the theoretical values $g(x_i)$ whether they are normally distributed with mean $E(\Delta y_i) = 0$, or not [16, 28–30]. For such an analysis the runs-test [9], the signs-test [31], the Wilcoxon-test [32] or the χ^2-test [33, 34] can be employed. Practical applications [cf. 16] and our own results, outlined in Section 3.1) give evidence that the four methods mentioned above may lead to contradictory results and, moreover, are not sensitive enough for identification of inadequate models.

To compare different models one may consider the weighted sums of least squares (WSLS), defined by

$$Q = \frac{1}{N}\sum_i w_i[y_i - g(x_i, \hat{\mathbf{p}})]^2, \quad i = 1, \ldots, N, \tag{1}$$

where w_i are weighting factors, $g(x_i, \hat{\mathbf{p}})$ is a regression function dependent on the vector of the ligand concentrations x_i and the WSLS-estimate of the vector of the kinetic parameters $\hat{\mathbf{p}} = (\hat{p}_1, \ldots, \hat{p}_M)$ and N is the number of measurements. For example, Otto *et al.* [35] performed a systematic discrimination of Monod-models for phosphofructokinase from red blood cells on the basis of the WSLS-values. Unfortunately, they (like most other authors using the WSLS-criterion) did not analyse whether the WSLS-values obtained for different models differed significantly.

If models of different order (i.e. different number of adjustable parameters) are taken into account, the variance ratio $Q/(N - M)$ is occasionally used instead of the WSLS-values [3, 18].

In particular cases where the models are nested, i.e. where model 1 (with a smaller number of parameters M_1) is just a submodel of model 2 (with a larger number of parameters M_2), the F-statistics

$$F = \frac{Q_1 - Q_2}{Q_2} \frac{N - M_2}{M_2 - M_1} \tag{2}$$

can be applied to assess whether the improvement in the fit from model 1 to model 2 is significantly better than would be expected by chance alone [36–39].

Further methods to compare either nested or nonnested models are based on the principle of parsimony; pick the model with the lowest number of parameters that fits the data best [40–42]. Both, the Akaike-information-criterion (AIC) [43–45] and the Schwarz-criterion (SC) [46] can be viewed as the sum of a fitting measure and a function proportional to the number of parameters:

$$\mathrm{AIC} = Q + 2M \tag{3}$$

and

$$\mathrm{SC} = Q + M \ln N. \tag{4}$$

Finally, a more sophisticated technique which requires considerable numerical effort to evaluate the goodness-of-fit is based on the investigation of several information criteria [47–50], e.g. Fishers information matrix, the elements of which are defined as second derivations of the WSLS-functional with respect to the parameters [51]

$$s_{ij} = \frac{\partial^2 Q}{\partial p_i \partial p_j}. \tag{5}$$

A lack of information with respect to a parameter is recognized by a quasi-singularity of this matrix [19]. Other approaches are based on Bayes-analysis [52–54].

All the above-mentioned tests have in common that they enable us to rule out unreliable models (exhibiting biased residuals, unrealistic WSLS-values, singularities of the information matrix etc.), but they do not allow us to decide whether a model can be viewed as an adequate model and whether differences between models are significant or not.

Recently, Zwanzig [55] proposed two novel statistical tests which can be employed to answer these questions. In this paper a brief outline of the basic idea of the Zwanzig-tests is given. Next, the methods are applied to numerically simulated kinetic data (Sections 3.1 and 3.2) as well as to real experimental data (Section 3.3).

2. THEORY

The observed data y_i are considered as realizations of the "true" kinetic model $f(x_i)$, i.e.

$$y_i = f(x_i) + e_i, \qquad i = 1, \ldots, N, \tag{6}$$

where the errors e_i are supposed to be randomly distributed with mean $E(e_i) = 0$ and variances $E(e_i^2) = \sigma_i^2$ known or estimated by s_i^2 from repetitions or additional experiments [33, 37, 56, 57]. The problem is to estimate $f(x)$ from a class of regression models $g_k(x, p)$ (the index k labels different models). In enzyme kinetics these models usually correspond to different reaction mechanisms.

The distance between the true model $f(x)$ and the regression function g_k is defined by

$$\| f - g_k \| = \frac{1}{N} \sum_i w_i [f(x_i) - g_k(x_i, p)]^2. \tag{7}$$

The specific form of equation (7) depends on the weights w_i and the experimental design x_i. The minimum distance

$$\Delta f_k = \min_{N \to \infty} \| f - g_k \| \tag{8}$$

is called the model error of model g_k. A given model is considered to be adequate if the model error Δf_k is zero i.e. if the best approximative function is equal to the true model in the asymptotic

limit. This strategy is equivalent to that of Kohn *et al.* [58] who assumed the total residual variance to be the sum of the variance of the experimental error and the bias-variance resulting from fitting an incorrect model to the data.

Since the true model f and the best approximative function are unknown they are replaced by the observations y_i and the WSLS-estimator $g_k(x_i, \hat{\mathbf{p}})$ [cf. equation (1)]. The resulting WSLS-functional Q_k converges towards Δf_k in the asymptotic limit.

For the special case of known variances σ_i^2 it can be shown that model g_k can be regarded to be adequate (hypothesis H: $\Delta f_k = 0$ is accepted) whenever

$$U_k < u_{1-\alpha}, \tag{9}$$

where the test statistics U_k is given by

$$U_k = \sqrt{\frac{N}{2}} \frac{Q_k - \frac{1}{N}\sum_i w_i \sigma_i^2}{\frac{1}{N}\sum_i w_i^2 \sigma_i^4}. \tag{10}$$

Here $u_{1-\alpha}$ denotes the $(1-\alpha)$-quantile of the normal distribution. By setting $w_i = \sigma_i^{-2}$, as should be done in order to obtain maximum likelihood estimations, equation (10) simplifies to

$$U_k = \sqrt{N/2}(Q_k - 1). \tag{11}$$

If σ_i^2 is unknown and has to be estimated by s_i^2, the hypothesis H: $\Delta f_k + \sigma^2 = s^2$ is tested by an analogous test replacing σ^2 by s^2 in equations (10) and (11).

A second test permits us to discriminate between two alternative models g_k and g_1 (hypothesis H: $\Delta f_k = \Delta f_1$; σ^2 known or estimated). The test statistic reads

$$T_{kl} = \sqrt{N/4}(Q_k - Q_1) t_{kl}^{-1/2} \tag{12a}$$

and

$$t_{kl} = \frac{1}{N}\sum_i w_i^2 \sigma_i^2 [g_k(x_i, \hat{p}_k) - g_1(x_i, \hat{p}_l)]^2. \tag{12b}$$

The following decision rule is valid:

$T_{kl} > u_{1-\alpha/2}$, choose g_1 (13a)

$|T_{kl}| \leqslant u_{1-\alpha/2}$, no significant difference between g_k and g_1 with respect to the data (13b)

$T_{kl} < -u_{1-\alpha/2}$, choose g_k. (13c)

It can be shown that the two tests (9) and (13a–c) have the asymptotic significance level α and are consistent.

3. RESULTS

3.1. Discrimination between a Michaelis–Menten-model and a Hill-model (simulated data, nested models)

A sigmoid velocity–substrate dependence is often phenomenologically described by the Hill-equation

$$g_{MM/H}(x) = \frac{v_{max} x^n}{K^n + x^n}, \qquad n \geqslant 1, \tag{14}$$

where v_{max} is the maximum rate, K is the half-saturation concentration and "Hill-n" determines the degree of sigmoidicity. Let us suppose that the rate equation (14) corresponds to the true kinetic model of the enzyme under consideration. If the sigmoidicity is weak (represented by a Hill-n which

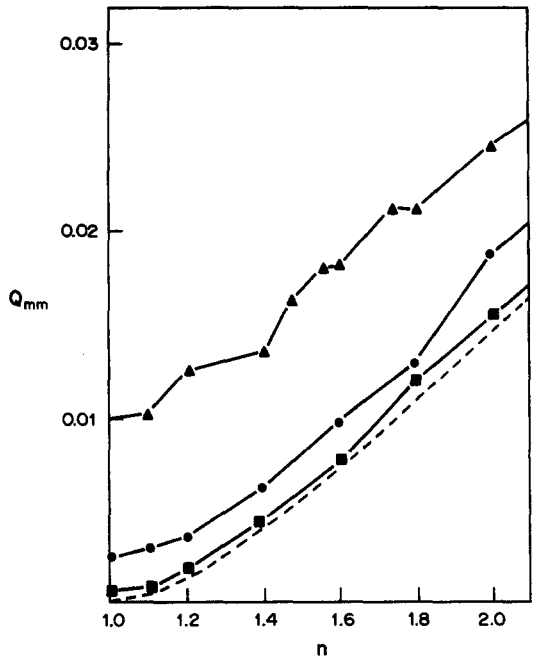

Fig. 1. WSLS-values Q obtained when fitting a Michaelis–Menten-model to data generated by a Hill-equation depending on Hill-n, for a constant absolute error of $0.025v_{max}$ (■), $0.05v_{max}$ (●) and $0.1v_{max}$ (▲). $N = 11$ logarithmic equidistant data were generated in the interval (0.1 K, 10 K). Each point corresponds to the mean of 20 repetitive simulations. The dashed line refers to the theoretically calculated model error Δf_{max} (i.e. $s = 0$).

is only slightly larger than unity), large experimental errors could make it impossible to assess whether the observed kinetic data are realizations of a sigmoidal model or of a pure hyperbolic rate law [Michaelis–Menten-model, equation (14) with $n = 1$]. Obviously, the models are nested since both models become identical for $n = 1$.

By several tests, we examined for different types of error above which critical value of n of the "true" Hill-model the Michaelis–Menten-model could be rejected.

In Fig. 1, the model error Δf_k and the error variance σ^2 are plotted as functions of Hill-n. Application of test (9) gives the U_k-values shown in Fig. 2(a). The WSLS-values obtained with the Hill-model were even smaller than the variance of the experimental errors (negative U_k-values) due to the partial elimination of the experimental errors by the regression procedure. Hence the Hill-model is accepted as an adequate model, regardless of the error. For a level of significance of $\alpha = 5\%$ ($u_{0,95} = 1.64$) the Michaelis–Menten–model has to be rejected as an adequate model for values of n larger than those given in Table 1.

These findings clearly demonstrate that even the incorrect Michaelis–Menten-model may provide U_k-values smaller than the critical one and, thus, has to be regarded to be adequate. As expected, the class of adequate models is enlarged with increasing error in the experimental data.

Although both models may serve under certain conditions as adequate data descriptions, the question remains whether the Hill-model provides a significantly better fit than the Michaelis–Menten-model. This question can be answered by the F-test for nested models [equation (2), Fig. 2(b)]. For the chosen examples we obtained the critical values of Hill-n shown in Table 1. We note that the degree of sigmoidicity (Hill-n) above which a discrimination between the true and the incorrect model is possible amounts to the same value for both statistical tests.

The above example demonstrates that the experimental errors of the used data set have to drop below a critical threshold in order to discriminate successfully between a hyperbolic and sigmoid rate law.

The results of a comparison between the U_k-test and several tests which are frequently used in the literature (χ^2-test, runs-test, signs-test and Wilcoxon-test) are given in Table 2.

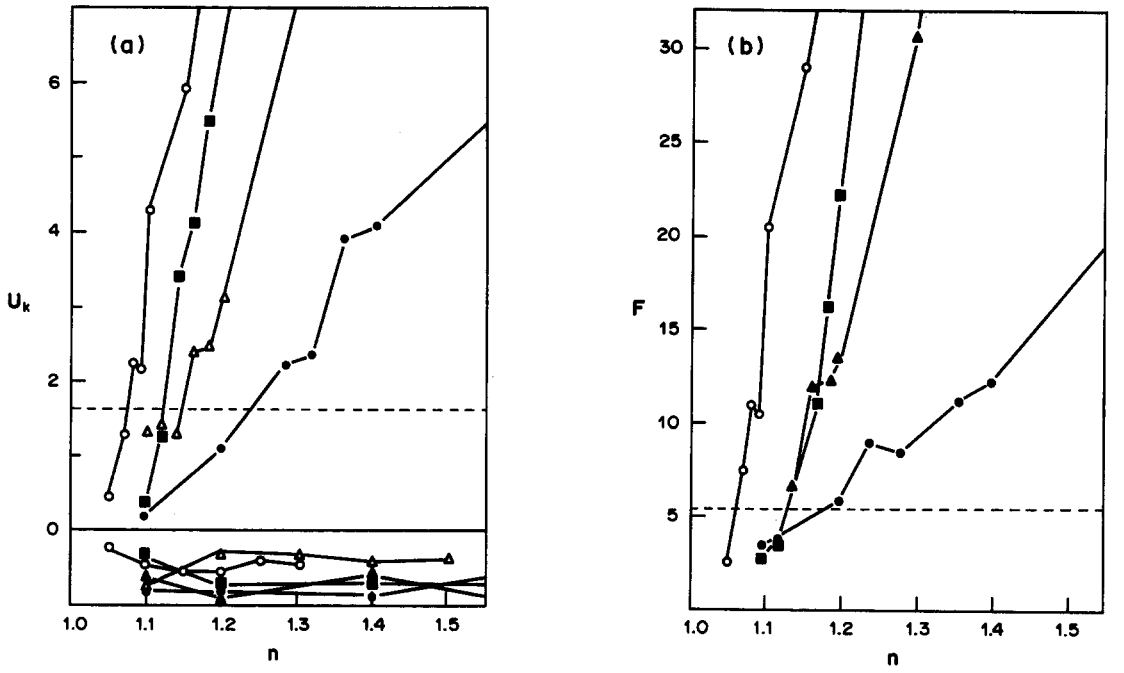

Fig. 2. Discrimination between the Michaelis–Menten-model and the Hill-model by the U_k-test (a) and F-test (b) depending on the Hill-n of the true model for different errors: constant absolute error of $0.025v_{max}$ (■) and $0.05v_{max}$ (●) and a constant relative error of 5% (●) and 10% (△), respectively. Each point corresponds to the mean of 20 repetitions. Dashed line: critical values for $\alpha = 5\%$.

3.2. Discrimination between competitive and noncompetitive inhibition (simulated data, nonnested models)

We discriminated between two rate laws, assuming competitive [equation (15a)] and noncompetitive [equation (15b)] inhibition of a two-substrate reaction dependent on the error and the experimental design. The example to be elucidated was proposed by Kohn *et al.* [58]:

$$g_1 = v_{max}\left[\left(\frac{K_{m1}}{S_1} + \frac{K_a}{S_1}\frac{K_{m2}}{S_2}\right)\left(\frac{I}{K_I} + 1\right) + 1 + \frac{K_{m2}}{S_2}\right]^{-1} \tag{15a}$$

and

$$g_2 = v_{max}\left[\left(\frac{K_{m1}}{S_1} + \frac{K_{m2}}{S_2}\left(\frac{K_a}{S_1} + 1\right) + 1\right)\left(\frac{I}{K_I} + 1\right)\right]^{-1}. \tag{15b}$$

The data were generated using g_1 as the true model and with constant relative errors between 2.5 and 15%. The U_k-test was applied to test the adequacy and the T_{kl}-test was used to distinguish between g_1 and g_2 (Fig. 3).

In the relevant ranges of experimental errors discrimination between the models was possible and the incorrect rate law could be rejected. Both tests yielded similar results. In all cases where the difference between the true and the incorrect model was significant the wrong model could be rejected, also. Nevertheless the fractional design was superior since it also provides information at

Table 1. Discrimination between Michaelis–Menten-model and Hill-equation; critical values of Hill-n above which the incorrect Michaelis–Menten-model can be rejected ($\alpha = 5\%$)

Error	U_k-test	F-test
Constant relative		
5%	1.08	1.06
10%	1.15	1.13
Constant absolute		
$0.025v_{max}$	1.12	1.13
$0.05v_{max}$	1.24	1.17

Table 2. Comparison between the U_k-test and several other goodness-of-fit criteria on the basis of the discrimination between the Michaelis–Menten-model and the Hill-model assuming a constant absolute error; critical values of Hill-n to reject the wrong Michaelis–Menten-model ($\alpha = 5\%$).

	Michaelis–Menten-model		Hill-model	
Error	$0.025v_{max}$	$0.05v_{max}$	$0.025v_{max}$	$0.05v_{max}$
U_k-test	1.12	1.24	ad.[a]	ad.
χ^2-test	1.7	2.5–3.0	ad.[b]	ad.[b]
Runs-test	1.5–1.7	1.7–2.4	ad.	ad.
Signs-test[c,d]	ad.	ad.	ad.	ad.
Wilcoxon-test[c]	ad.	ad.	ad.	ad.

[a] Adequate model up to $n \leqslant 4.0$.
[b] But rejection of the true model in a considerable number of simulations assuming constant relative errors.
[c] No differences between both models up to $n \leqslant 4.0$.
[d] No dependence of the test quantities on Hill-n up to $n \leqslant 4.0$.

points where both substrates are at a low level, while the univariate design with $N = 18$ was insufficient in the presence of larger errors. Moreover, the univariate designs yielded poor estimates of the parameter values. Our results are in a good agreement with those of Kohn *et al.* [58].

3.3. Application of the proposed method to assess a kinetic model of malarial phosphofructokinase

As in man, phosphofructokinase is an important glycolytic control enzyme in malarial parasites. Therefore the kinetic characterization of this enzyme is an essential step in gaining a deeper understanding of the energy metabolism of the parasites. It has to be emphasized that our aim was not to elucidate the real reaction mechanism of the enzyme (which would require additional investigations) but to obtain a good phenomenological description of its kinetic properties.

The kinetic measurements were carried out with stroma-free lysates of the parasites isolated from red blood cells of *Plasmodium berghei* infected mice. PFK-activity was measured at pH 6.5, 6.8 and 7.2, respectively. The substrate concentrations were varied between 0.03–3.0 mM F6P

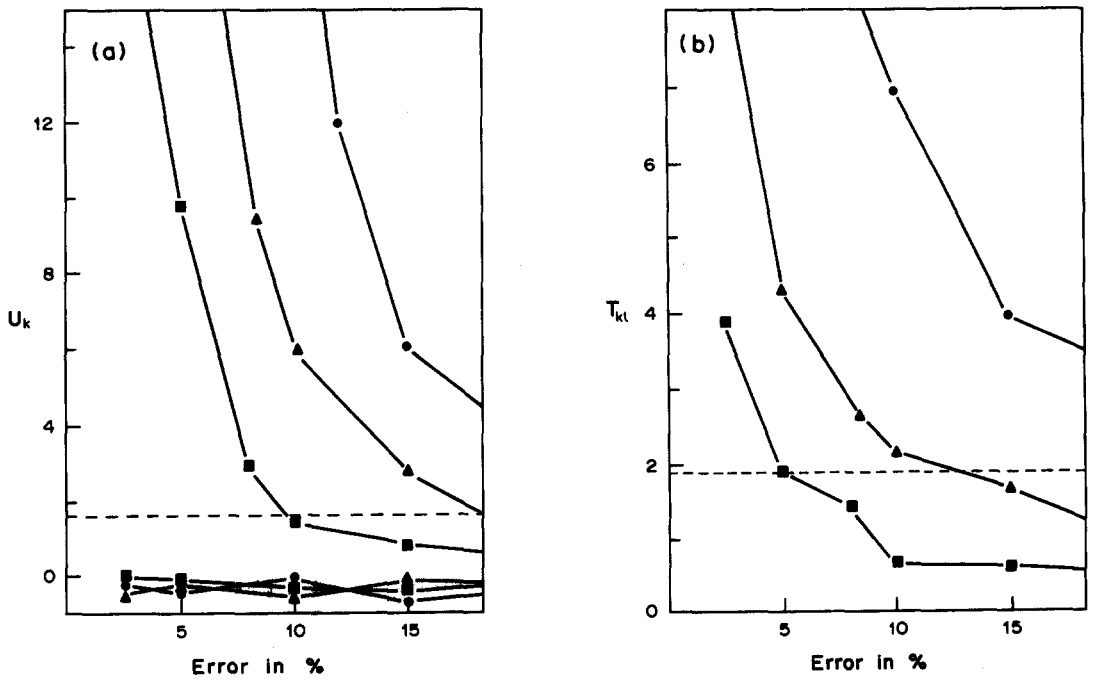

Fig. 3. Discrimination between competitive and noncompetitive inhibition by the U_k-test (a) and T_{kl}-test (b) depending on the error for different experimental designs. (●) Fractional design: all ligand concentrations were varied simultaneously, $N = 64$. (▲) Univariate design: only one substrate- and the inhibitor-concentration were varied at a time while the other substrate concentration is held constant at saturation level, and vice versa, $N = 60$. (■) Univariate design, $N = 18$. Dashed line: critical values for $\alpha = 5\%$.

and 0.04–4.8 mM ATP (for details see Ref. [59]). Repetitional measurements and additional investigations indicated a constant experimental error which was estimated to be $s = 0.035\, v_{max}$. The weights used in all calculations were $w_i = s_i^{-2}$.

Owing to the complex kinetic behaviour of the enzyme, several kinetic models of different complexity were fitted to the $N = 172$ data by a WSLS-procedure assuming different reaction mechanisms and modes of allosteric regulation. Common statistical tests for checking the goodness-of-fit as well as the usual kinetic plots [60, 61] failed or were insufficient for model discrimination. After the rejection of obviously unsuitable rate laws six models were involved in model discrimination.

By application of the T_{kl}-test [equations (13a–c)], the differences between three models, denoted a3, a4 and c4 in Ref. [59], were found to be not significant at a level of $\alpha = 5\%$, i.e. these three rate laws are equally well-suited to describe the data (Fig. 4). The fits of other models with the same (model b4) or a smaller (model a1) number of parameters were worse. But by application of the U_k-test [equation (9)] all rate laws except model a4 [equation (17)] could be rejected:

$$v = \frac{v_{max}\,\mathrm{MgATP}\,\mathrm{F6P}}{(\mathrm{MgATP} + K_{ma})(\mathrm{F6P} + K_f)} \cdot \frac{1}{1 + L_0 \left[\dfrac{\left(1 + \dfrac{H^+}{K_{h1}}\right)\left(1 + \dfrac{H^+}{K_{h2}}\left(1 + \dfrac{\mathrm{MgATP}}{K_{ma1}} + \dfrac{\mathrm{ATP}}{K_{a1}}\right)\right)}{1 + \dfrac{H^+}{K_{h1}} + \dfrac{\mathrm{F6P}}{K_{f1}}} \right]^n}. \tag{16}$$

This favoured model, assumes a rapid-equilibrium random mechanism and allosteric inhibition by ATP and activation by F6P. By additional investigations, K_{ma}, K_f, L_0 and n were shown to be pH-independent. Thus, only the dependence of the allosteric activation and inhibition on pH were considered by assuming two virtual protonation steps: ATP binds only to its protonated site and F6P binds only to its unprotonated site (Fig. 5, parameters given in Table 3).

Although the proposed model of malarial PFK gives no proof for the real mechanism of the enzyme, it provides an adequate description of the kinetic properties in the relevant ranges of substrate concentrations.

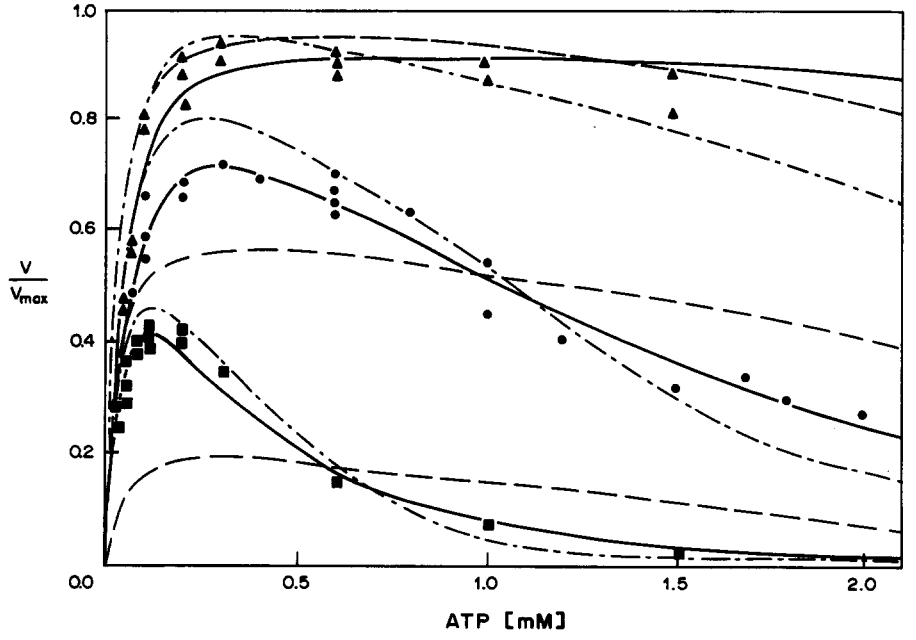

Fig. 4. Dependence of PFK-activity on ATP at pH 7.2 (▲), 6.8 (●) and 6.5 (■), respectively for [F6P] = 1.0 mM. The fits correspond to model a4 (——), model a1 (— —) and model b4 (— · —).

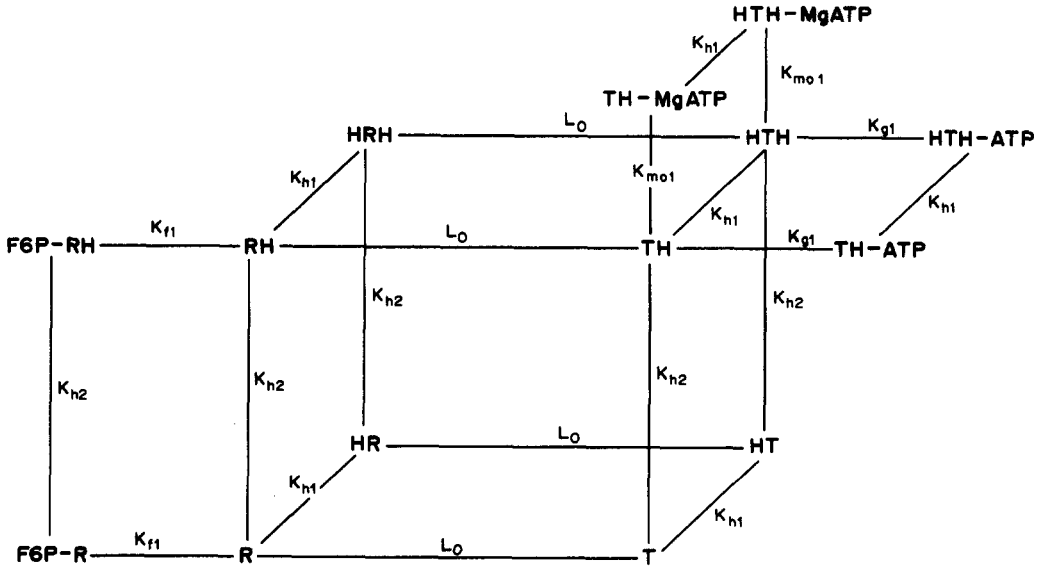

Fig. 5. Proposed kinetic model for the allosteric regulation of malarial phosphofructokinase (model a4). All complexes containing "R" are enzymatically active while those with "T" are inactive.

4. DISCUSSION

The two novel statistical tests for model discrimination outlined in this paper may help to tackle the two principal problems which arise when setting up a model:

(1) Is the chosen model adequate, i.e. does it provide a satisfactory quantitative description of the data which it is based on?
(2) Is it possible to select from a class of several models the best one, i.e. the model which provides a singificantly better fit than the others?

The first question can be answered by means of the U_k-test and the second by the T_{kl}-test.

The crucial task in application of these tests is to estimate the error variance, which can be done by additional data as wall as during the regression procedure from repetitional measurements (robust regression [62]). The structure of the measurement errors depends on the experimental conditions [63–69]. While it was reasonable to assume a constant absolute error for the kinetic data of malarial PFK, we found an error of a mixed type,

$$s_i^2 = s_1^2 + s_2^2 y_i^2, \tag{17}$$

in the case of a Michaelis–Menten kinetics (unpublished data).

It should be noted that both methods are based on asymptotic properties. Thus, the number of parameters is not considered. The models to be compared should not differ too much in their number of parameters, and a sufficiently large number of data should be available.

At least for the chosen examples, the novel tests are demonstrated to be more sensitive than other commonly used criteria to indicate unsatisfactory models. This is in agreement with the fact that nonparametric procedures are, in general, less powerful than parametric methods.

Table 3. Phosphofructokinase from *Plasmodium berghei*: estimated parameter values of model a4

K_{ma}	0.031 mM
K_f	0.033 mM
K_{a1}	0.023 mM
K_{ma1}	0.090 mM
K_{f1}	0.064 mM
K_{h1}	0.019 μM
K_{h2}	3.16 μM
L_0	3.55
n	3.79

The proposed tests are simple and can easily be implemented within a least-squares regression procedure. Nevertheless, we share the opinion of Atcins [16] that one should make use of several tests to assess the goodness-of-fit and not rely on only one such method. We wish to stress that mathematical procedures alone can never substitute for poor and unreliable experiments. A critical examination of discrepancies between the model and data must always include possible defects of the data (structure of experimental errors, detection of outliers [70, 71] etc.), defects of the model (model discrimination) and defects of the experimental design leading to a lack of information with respect to certain parameters of the model (optimization of the experimental design [72–77]).

REFERENCES

1. G. N. Wilkinson, Statistical estimations in enzyme kinetics. *Biochem. J.* **80,** 324–332 (1961).
2. F. Acton, *Numerical Methods that Work.* Harper & Row, New York (1970).
3. Y. Bard, *Nonlinear Parameter Estimation.* Academic Press, New York (1974).
4. C. Daniel and F. S. Wood, *Fitting Equations to Data,* 2nd edn. Wiley, New York (1974).
5. J. V. Beck and K. J. Arnold, *Parameter Estimation.* Wiley, New York (1977).
6. R. I. Jennich and M. L. Ralston, Fitting nonlinear models to data. *A. Rev. Biophys. Bioeng.* **8,** 195–209 (1979).
7. D. G. Watts, An introduction to nonlinear least squares. In *Kinetic Data Analysis: Design and Analysis of Enzyme and Pharmacokinetic Experiments* (Ed. L. Endrenyi), pp. 1–24. Plenum Press, New York (1981).
8. D. M. Metzler, Statistical properties of kinetic estimates. In *Kinetic Data Analysis: Design and Analysis of Enzyme and Pharmacokinetic Experiments* (Ed. L. Endrenyi), pp. 25–37. Plenum Press, New York (1981).
9. N. R. Draper and H. Smith, *Applied Regression Analysis.* 2nd edn. Wiley, New York (1981).
10. R. E. Maine and K. W. Iliff, Formulation and implementation of a practical algorithm for parameter estimation with process and measurement noise. *SIAM Jl appl. Math.* **41,** 558–579 (1981).
11. R. G. Duggleby, A nonlinear regression program for small computers. *Analyt. Biochem.* **110,** 9–18 (1981).
12. D. A. Ratkowsky, *Nonlinear Regression Modelling.* Dekker, New York (1983).
13. H. Bunke and O. Bunke, Das empirische Entscheidungsprinzip und die Wahl von Regressionmodellen. *Biomt. Z.* **16,** 167–184 (1974).
14. L. Breiman and D. A. Freedman, How many variables should be entered in a regression equation? *J. Am. statist. Ass.* **78,** 131–136 (1983).
15. P. J. Kempthrone, Admissible variable-selection procedures when fitting regression models by least squares for prediction. *Biometrika* **71,** 593–597 (1984).
16. G. L. Atcins, Tests for the goodness of fit of models. *Biochem. Soc. Trans.* **4,** 357–361 (1976).
17. E. M. Landaw, Optimal multilcompartmental sampling designs for parameter estimation. *Math. Comput. Simuln* **24,** 525–530 (1982).
18. E. M. Landaw and J. J. Distefano III, Multiexponential, multicompartmental and noncompartmental modelling. II Data analysis and statistical considerations. *Am. J. Physiol.* **246,** R665–R677 (1984).
19. J. G. Reich, G. Wangermann, M. Falck and K. Rohde, A general strategy for parameter estimation from isosteric and allosteric kinetic data and binding measurements. *Eur. J. Biochem.* **26,** 368–379 (1972).
20. M. J. Box, Bias in nonlinear estimation. *J. R. Statist. Soc.* **B32,** 171–201 (1971).
21. A. C. Atcinson, Posterior probabilities for choosing a regression model. *Biometrika* **65,** 39–48 (1978).
22. K. J. Ellis and R. G. Duggleby, What happens when data are fitted to the wrong equation. *Biochem. J.* **171,** 513–517 (1978).
23. E. Läärä and J. N. S. Matthews, The equivalence of two models for ordinary data. *Biometrika* **72,** 206–207 (1985).
24. B. Mannervik and T. Bártfai, Discrimination between mathematical mdoels of biochemical systems exemplified by enzyme steady state kinetics. *Acta biol. med. germ.* **31,** 203–215 (1973).
25. B. Mannervik, Nonlinear regression methods in design of experiments and mathematical modelling. Applications of the analysis of the steady state kinetics of glutathione reductase. *Biosystems* **7,** 107–119 (1975).
26. B. Mannervik, Regression analysis, experimental error, and statistical criteria in the design and analysis of experiments for discrimination between rival kinetic models. *Meth. Enzym.* **87,** 370–390 (1982).
27. J. B. Pedersen and S. M. Pedersen, A general method of deriving the best binding site model consistent with experimental binding data. *Biophys. Chem.* **22,** 255–262 (1985).
28. D. R. Cox and E. J. Snell, A general definition of residuals. *J. R. statist. Soc.* **B30,** 248–275 (1968).
29. R. D. Cook and S. Weisberg, *Residuals and Influence in Regression.* Chapman & Hall, New York (1982).
30. R. D. Cook and C. L. Tsai, Residuals in nonlinear regression. *Biometrika* **72,** 23–29 (1985).
31. S. Siegel, *Nonparametric Statistics,* p. 254. McGraw-Hill, London (1956).
32. S. Wilcoxon, *Some Rapid Approximative Statistical Procedures.* Cyamind Co., New York.
33. J. H. Ottaway, Normalizatin in the fitting of data by iterative methods. *Biochem. J.* **134,** 729–736 (1973).
34. G. Hirotsu, Cumulative chi-squared statistic as a tool for testing the goodness of fit. *Biometrika* **73,** 165–173 (1986).
35. M. Otto, R. Heinrich, B. Kühn and G. Jacobasch, A mathematical model of the influence of F6P, ATP, K^+, ammonium, and Mg^{2+} on the phosphofructokinase from rat erythrocytes. *Eur. J. Biochem.* **49,** 169–178 (1974).
36. R. G. Duggleby and D. T. Dennis, NAD-specific glycerin-aldehyde-3-phosphate dehydrogenase from *Pisum sativum. J. biol. Chem.* **249,** 167–174 (1974).
37. C. N. McMinn and J. H. Ottaway, Studies on the mechanism and kinetics of the 2-oxoglutarate dehydrogenase system from pig heart. *Biochem. J.* **161,** 569–581 (1977).
38. W. G. Bardsley, Model discrimination, conditions when the correct model can be identified for transient, steady-state and binding data. In *Dynamics of Biochemical Systems* (Eds S. Damjanovich, T. Keleti and L. Trón), pp. 267–287. Academic Press, New York (1985).

39. J. F. Harper, Precise calculations of the cumulative distribution function and its inverse function for Fishers F- and Students t-test. *Comput. Meth. Prog. Biomed.* **21,** 127–129 (1985).
40. C. L. Mallows, Some comments on C_p. *Technimetrics* **15,** 661–675 (1973).
41. C. J. Stone, Admissible selection of an accurate and parsimonious normal linear regression model. *Ann. Statist.* **9,** 475–485 (1981).
42. A. Y. C. Kuk, All subsets regression in a proportional hazards model. *Biometrika* **71,** 587–592 (1984).
43. H. Akaike, A new look at the statistical model identification. *IEEE Trans. autom. Control.* **AC19,** 716–723 (1974).
44. M. Stone, An asymptotic equivalence of choice of model by cross-validation and Akaike's criterion. *J. R. statist. Soc.* **B39,** 44–47 (1977).
45. H. Akaike, A Bayesian analysis of the minimum AIC procedure. *Ann. Inst. Statist. Math.* **A30,** 9–14 (1978).
46. G. Schwarz, Estimating the dimension of a model. *Ann. Statist.* **6,** 461–464 (1978).
47. M. Stone, Application of a measure of information to the desigtn and comparison of regression experiments. *Ann. Math. Statist.* **39,** 55–72 (1959).
48. D. M. Borth, A total entropy criterion for the dual problem of model discrimination and parameter estimation. *J. R. statist. Soc.* **B37,** 77–87 (1975).
49. A. C. Atcinson, A note on the generalized information criterion for choice of a model. *Biometrika* **67,** 413–418 (1980).
50. H. Akaike, Likelihood of a model and information criteria, *J. Econometr.* **16,** 3–14 (1981).
51. M. Markus and Th. Plesser, Design and analysis of progress curves in enzyme kinetics. *Biochem. Soc. Trans.* **4,** 361–364 (1976).
52. K. M. Garer and M. S. Geisel, Discriminating among alternative models: Bayesian and non-bayesian methods. In *Frontiers of Economics* (Ed. P. Zarembka), pp. 49–80. Academic Press, New York (1974).
53. A. F. M. Smith and D. J. Spiegelhalter, Bayes factors and choice criteria for linear models. *J. R. statist. Soc.* **B42,** 213–220 (1980).
54. L. R. Pericchi, An alternative to the standard bayesian procedure for discrimination between normal linear models. *Biometrika* **71,** 575–586 (1984).
55. S. Zwanzig, The choice of approximative models in non-linear regression. *Math. Opsforsch. Statist. Ser. Statist.* **11,** 23–47 (1980).
56. A. C. Storer, M. G. Darlison and A. Cornish-Bowden. The nature of experimental error in enzyme kinetic measurements. *Biochem. J.* **151,** 361–367 (1975).
57. B. Mannervik, I. Jacobson and M. Warholm, A new procedure to derive weighting factors for nonlinear regression analysis applied to enzyme kinetic data. *Biochim. biophys. Acta* **567,** 43–48 (1979).
58. M. C. Kohn, L. E. Menten and D. Garfinkel, A convenient computer program for fitting enzymatic rate laws to steady-state data. *Computers biomed. Res.* **12,** 461–469 (1979).
59. D. Buckwitz, G. Jacobasch, C. Gerth, H.-G. Holzhütter and R. Thamm, A kinetic model of phosphofructokinase from *Plasmodium berghei*. Influence of ATP and fructose-6-phosphate. *Molec. Biochem. Parasit.* **27,** 225–232 (1988).
60. M. Dixon and E. Webb, *Enzymes*, 2nd edn, pp. 116–145. Academic Press, New York (1964).
61. W. W. Cleland, Steady state kinetics. In *The Enzymes*, 3rd edn, Vol. III, *Kinetics and Mechanism* (Ed. P. D. Boyer), pp. 1–65. Academic Press, New York (1970).
62. W. R. Porter and W. F. Trager, Improved non-parametric statistical methods for the estimation of Michaelis–Menten kinetic parameters by the direct linear plot. *Biochem. J.* **161,** 293–302 (1977).
63. A. Cornish-Bowden and R. Eisenthal, Estimation of Michaelis-constant and maximum velocity from the direct linear plot. *Biochim. biophys. Acta* **523,** 268–272 (1978).
64. A. Cornish-Bowden and L. Endrenyi, Estimation of enzyme kinetic parameters without prior information about the weights or error distribution. In *Dynamics of Biochemical Systems* (Eds S. Damjanovich, T. Keleti and L. Trón), pp. 309–322. Academic Press, New York (1985).
65. J. H. Ottaway and D. K. Apps, Some examples of the use of computer-produced contour plots in the fitting of enzyme rate equations to reaction-velocity measurements. *Biochem. J.* **130,** 861–870 (1972).
66. P. Askelöf, M. Korsfeldt and B. Mannervik, Error structure of enzyme kinetic experiments—implications for weighting in regression analysis of experimental data. *Eur. J. Biochem.* **69,** 61–67 (1976).
67. L. A. Stefanski, The effects of measurement error on parameter estimation. *Biometrika* **72,** 583–592 (1985).
68. R. Tommasini, L. Endrenyi, P. A. Taylor, D. J. Mahuran and J. A. Lowden, A statistical comparison of parameter estimation for the Michaelis–Menten kinetics of human placental hexaminidase. *Can. J. Biochem. cell. Biol.* **63,** 225–230 (1985).
69. B. Mannervik and H. Danielson, Empirical error functions for model discrimination and parameter estimation in enzyme kinetics. In *Dynamics of Biochemical Systems* (Eds S. Damjanivich, T. Keleti and L. Trón), pp. 323–337. Academic Press, New York.
70. R. Nical, P. Smith and P. Raggatt, A robust microcomputer routine for the identification of outlying and influential points in radioimmunoassays. *Computers biomed. Res.* **18,** 334–346 (1985).
71. D. M. Rocke, Outlier resistance in small samples. *Biometrika* **73,** 175–181 (1986).
72. A. C. Atkinson and D. R. Cox, Planning experiments for discrimination between models. *J. R. statist. Soc.* **B36,** 321–348 (1974).
73. P. D. H. Hill, A review of experimental design procedures for regression and model discrimination. *Technometrics* **20,** 15–21 (1978).
74. L. Endrenyi (Ed.), Design of experiments for estimating enzyme and pharmacokinetic parameters. In *Kinetic Data Analysis: Design of Experiments and Analysis of Enzyme and Pharmacokinetic Experiments*, pp. 137–167. Plenum Press, New York (1981).
75. G. Herrenhöfer and D. Rasch, Experimental design and optimal decision, I general remarks. *Math. Opsforshc. Statist. Ser. Statist.* **11,** 115–123 (1980).
76. B. D. Roberts and K. E. Ebener, Steady state enzyme kinetics: experimental design and data analysis by microcomputer. *Int. J. biomed. Comput.* **15,** 433–441 (1984).
77. E. Caneld, A microcomputer method for designing optimal experiments. *Int. J. biomed. Comput.* **16,** 257–166 (1985).